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Title:	Reconfiguring the metabolism of photosynthetic microbes for their development as biotechnological platforms
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Issued:	2021-08-12

Seminar- Cemvita Factory Inc.

Reconfiguring the metabolism of photosynthetic microbes for their development as biotechnological platforms

Raul Gonzalez

8/13/2021

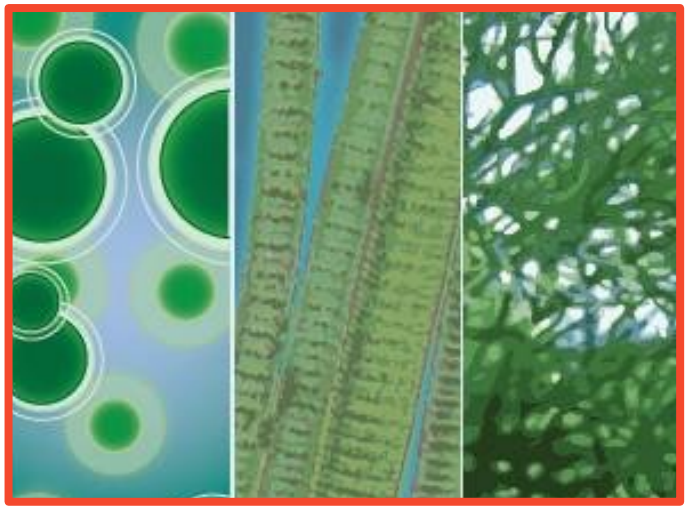
Utilization of the marine biodiversity to foster technological innovation

Bioluminescence
(i.e. GFP)

Improved fisheries
(i.e. Atlantic Salmon)

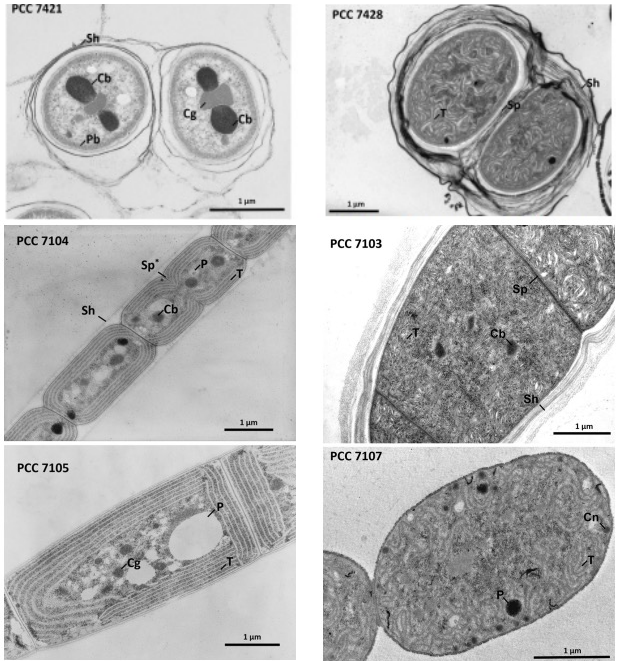
Bioremediation
(i.e. *Alcanivorax*)

Bioactive compounds
(Limulus Amoebocyte
Lysate)

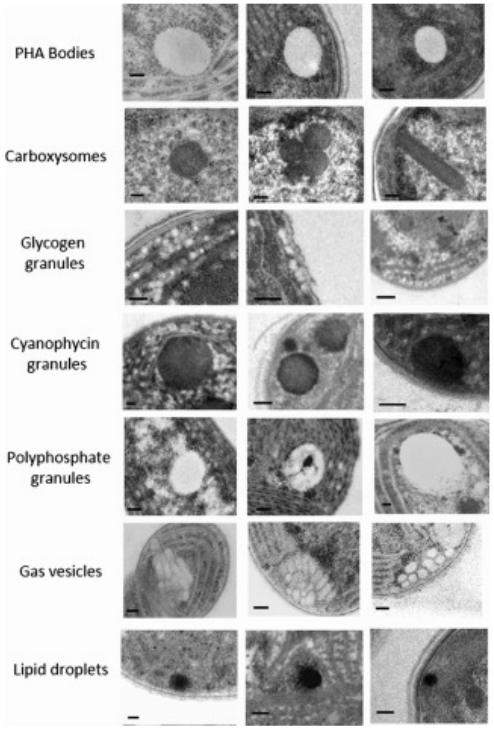


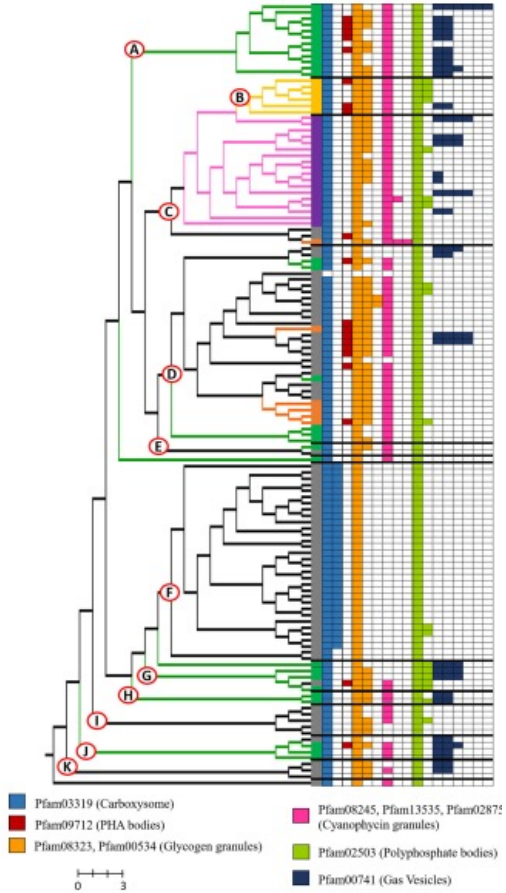
Algal Biofuels and Bioproducts

Cyanobacterial ultrastructure and its relation to genomic features (Pfams)



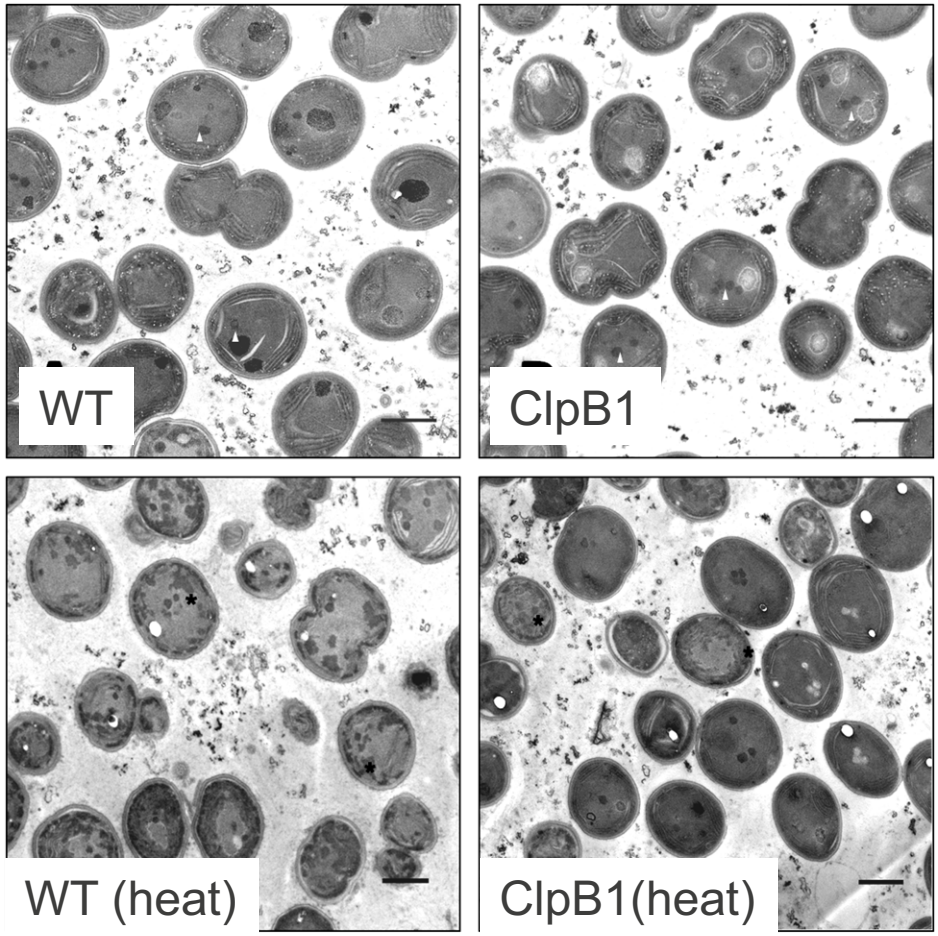
Photosynth Res (2016) 129:147–157

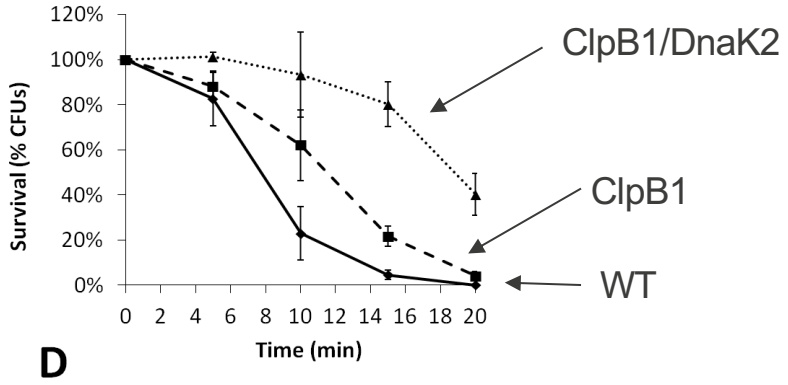


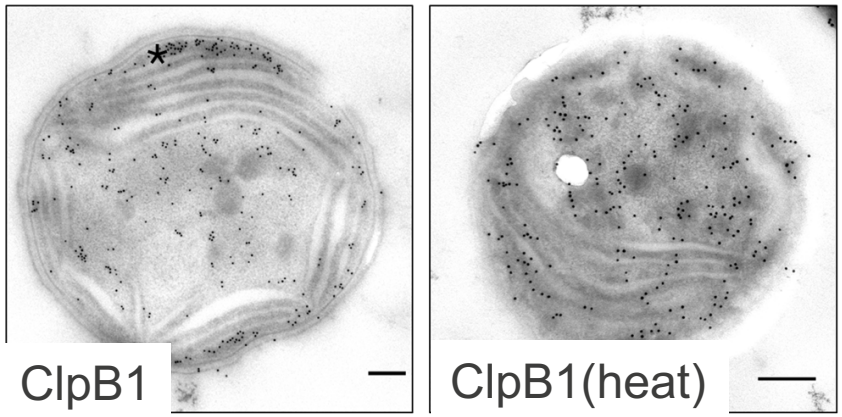


Improve environmental stress tolerance

Increase tolerance to heat stress as a proxy for other stresses

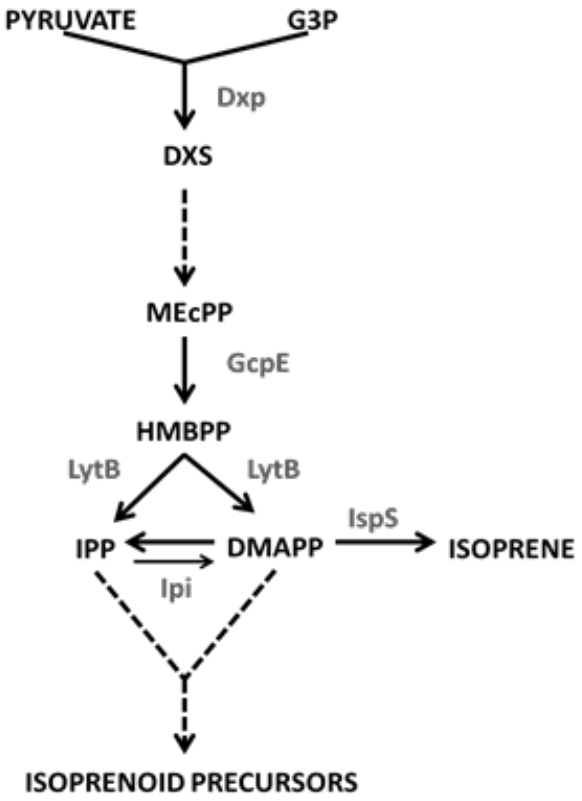


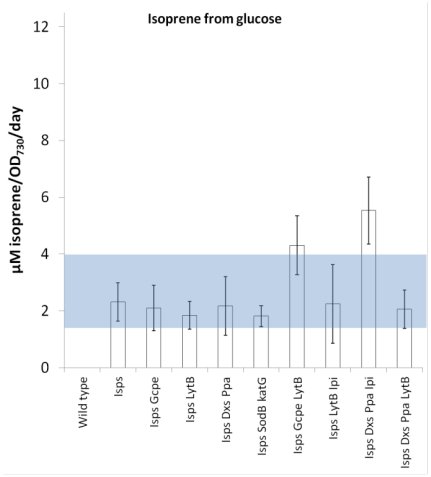


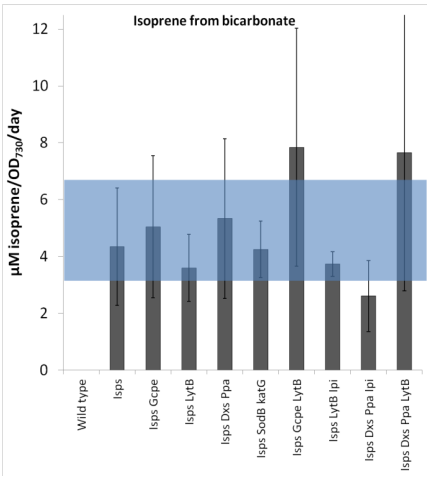


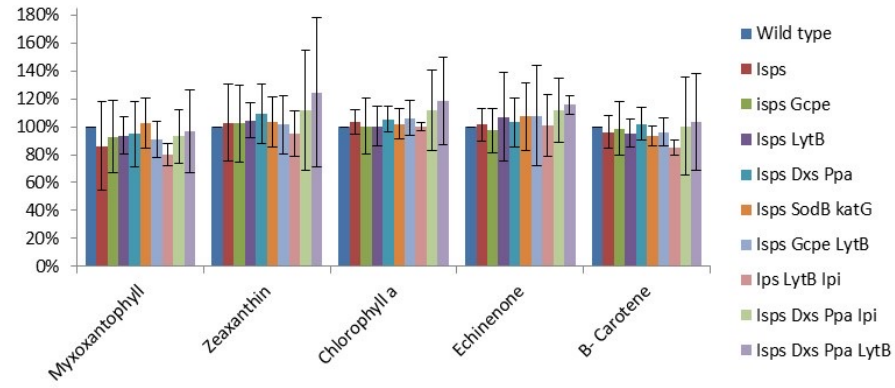
Increase Product Output

Increase yields of an isoprene-evolving mutant via pathway engineering

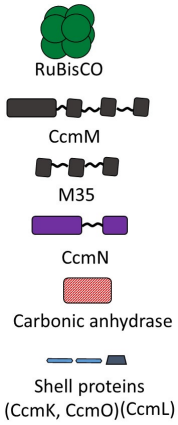


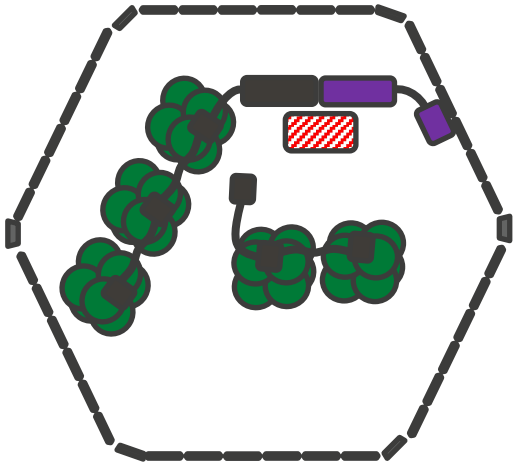




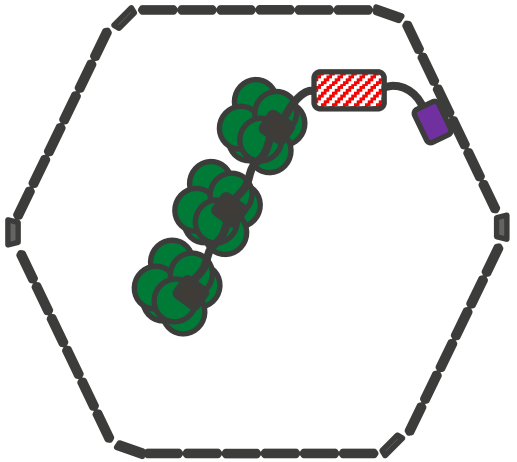


Improve CO_2 fixation machinery
Streamlined assembly of carboxysomes





Wildtype carboxysome

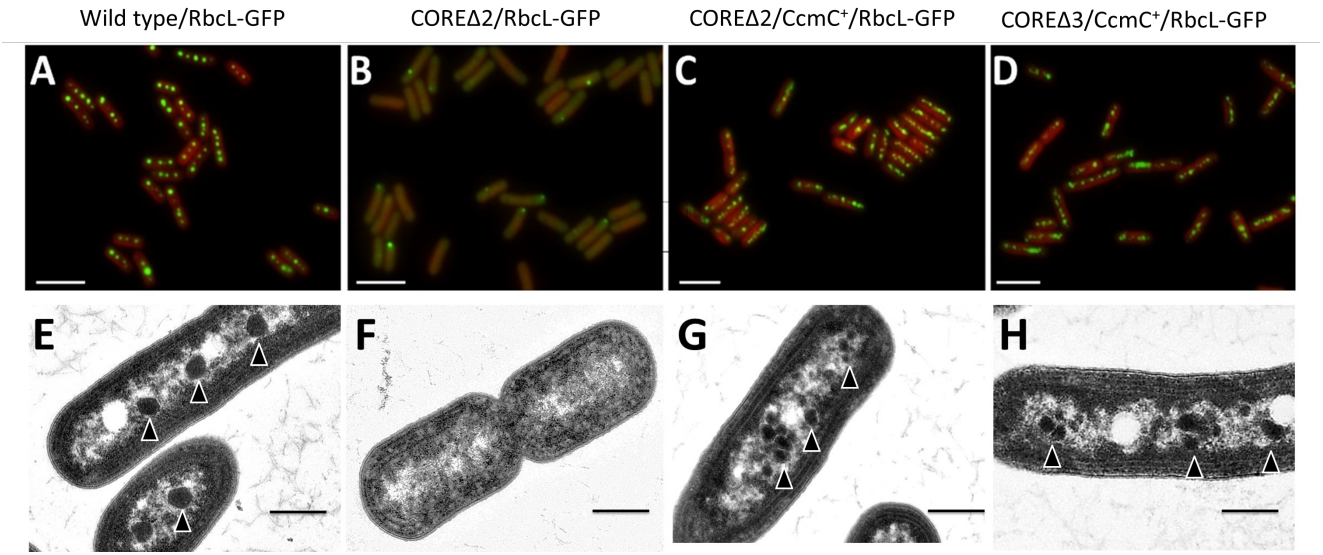


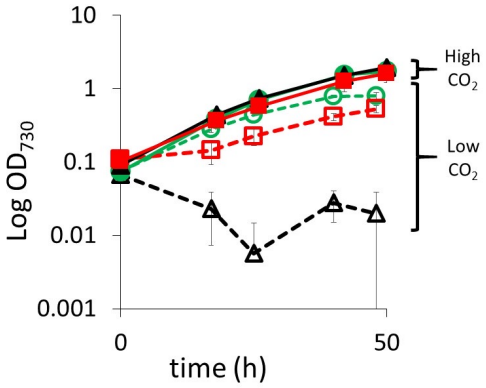
Streamlined carboxysome

- Flexibility of subunits under environmental conditions
- Several subunits required for transfer to other organisms
- Complex assembly and regulation in other organisms

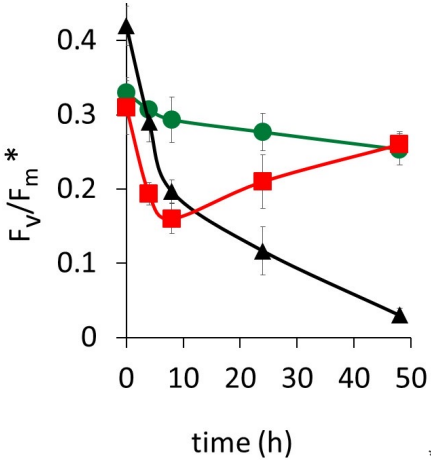
- Fixed stoichiometry of the composing domains
- Increased portability (fewer number of proteins required)
- Simplified assembly and regulation (one core assembly protein) in other organisms
- Potential for improvement

Improve CO_2 fixation machinery
Streamlined assembly of carboxysomes





Wild type/RbcL-GFP
COREΔ2/RbcL-GFP
COREΔ3/CcmC/RbcL-GFP

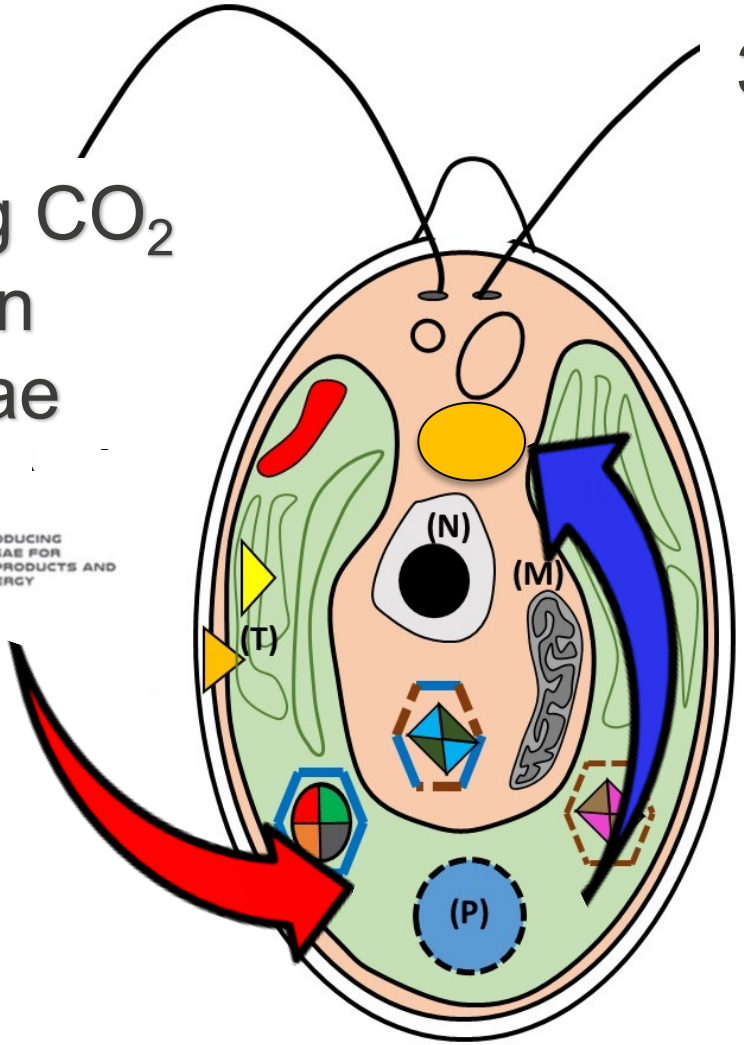


* Upon change from 5% CO_2 to air

Research at LANL

1) Increasing CO_2 fixation in microalgae

PACE PRODUCING ALGAE FOR COPRODUCTS AND ENERGY



3) Customized PHA production in cyanobacteria

LDRD Los Alamos NATIONAL LABORATORY EST. 1943

2) Increasing carbon storage compounds in microalgae

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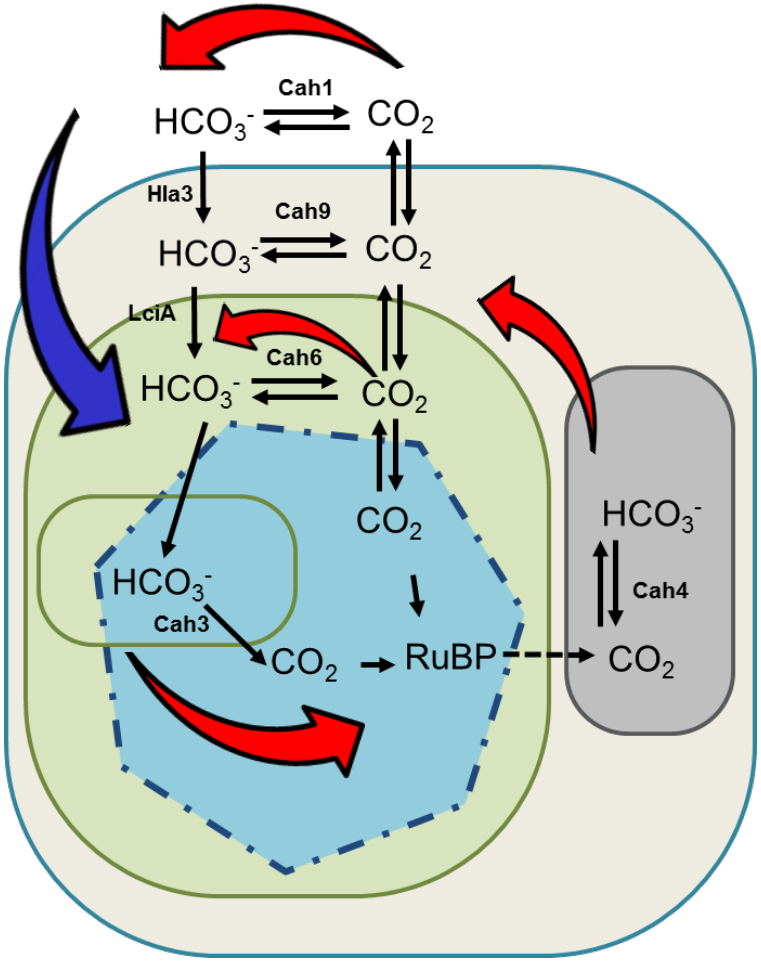
BIOENERGY TECHNOLOGIES OFFICE

Increasing carbon flux towards RuBisCO



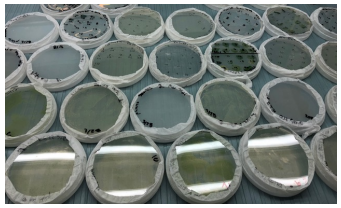
Carbonic anhydrase overexpression:

- ***CAH1*** *and* ***CAH6****: Shift the equilibrium towards bicarbonate (decrease CO_2 leakage).*
- ***CAH3:*** *Produce more CO_2 available for RuBisCO.*
- ***CAH4****: Mobilize bicarbonate pool from mitochondria to the CCM.*
- ***BCA:*** *Shown previously to increase photosynthetic efficiency in plants.*

HCO_3^- transporter overexpression:

- ***HLA3 and LCIA:*** *Increase the bicarbonate flux towards the chloroplast.*

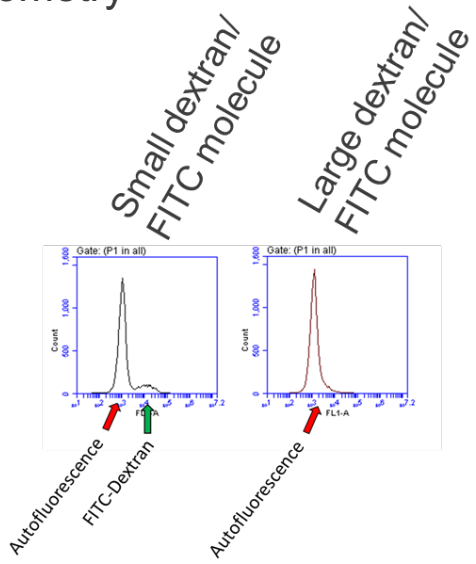
Challenge overcome: Development of a transformation method for *Chlorella sorokiniana*



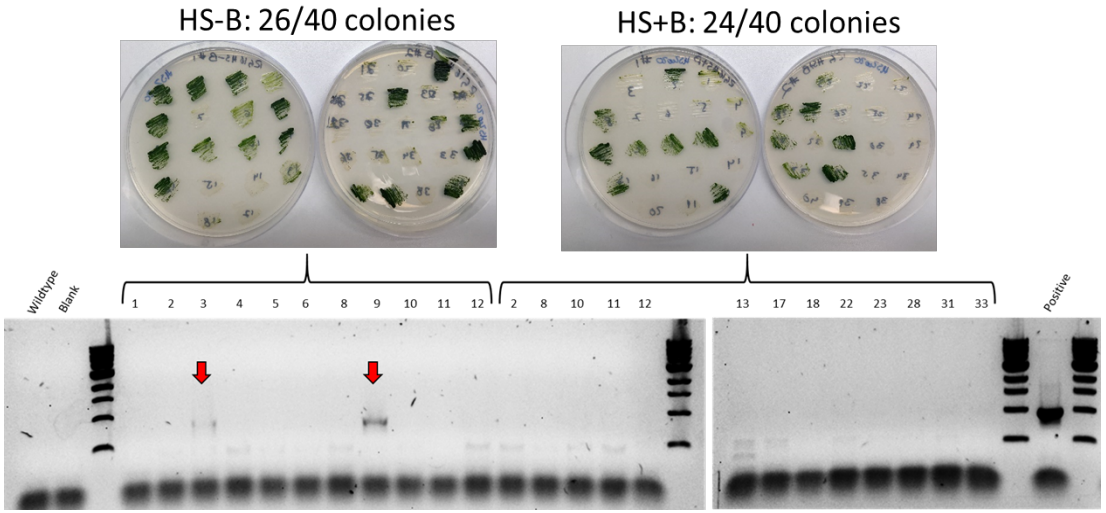
- Biolistic DNA delivery and antibiotic selection frequently yielded too many false positives. Screening *practically* impossible.

✓ Electroporation, Flow cytometry and Physiology

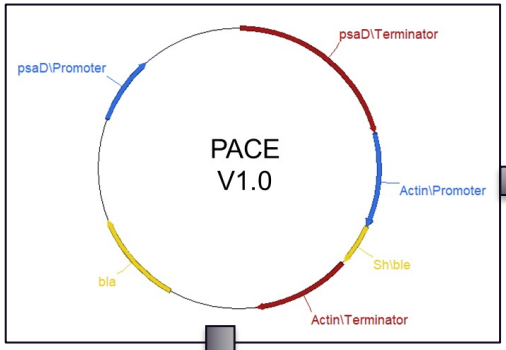
➢ Delivered fluorescent molecules by electroporation, screened by flow cytometry

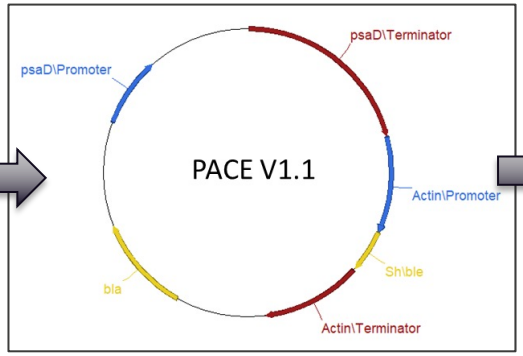


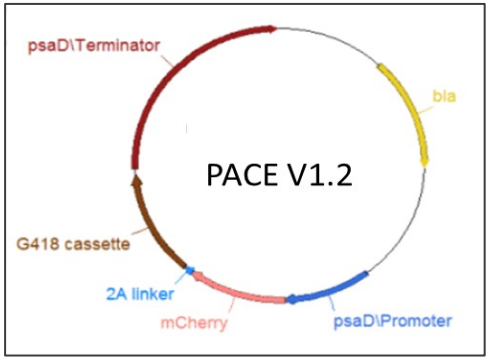
➢ Screened different culture conditions for recovery, selective pressure.

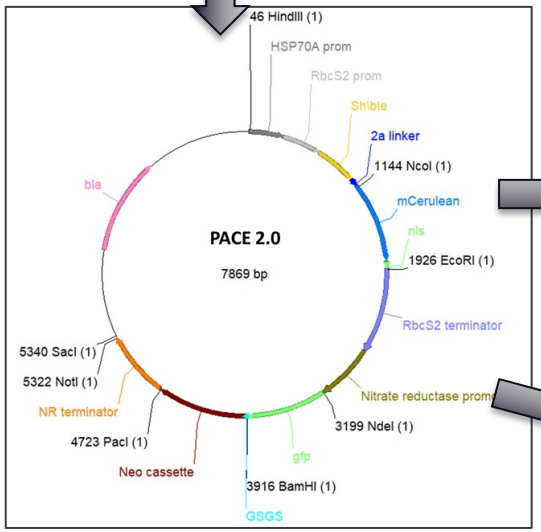


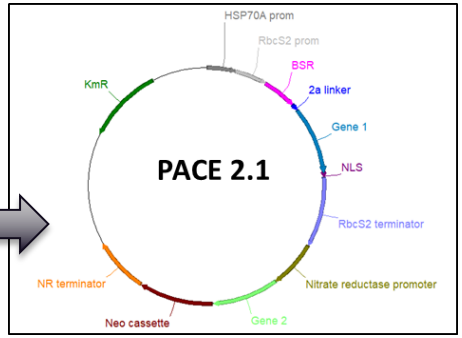
LANL team pushes boundaries of the state-of-technology

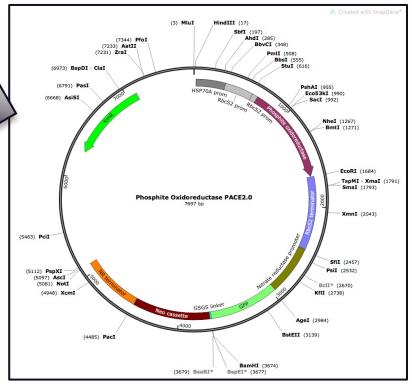












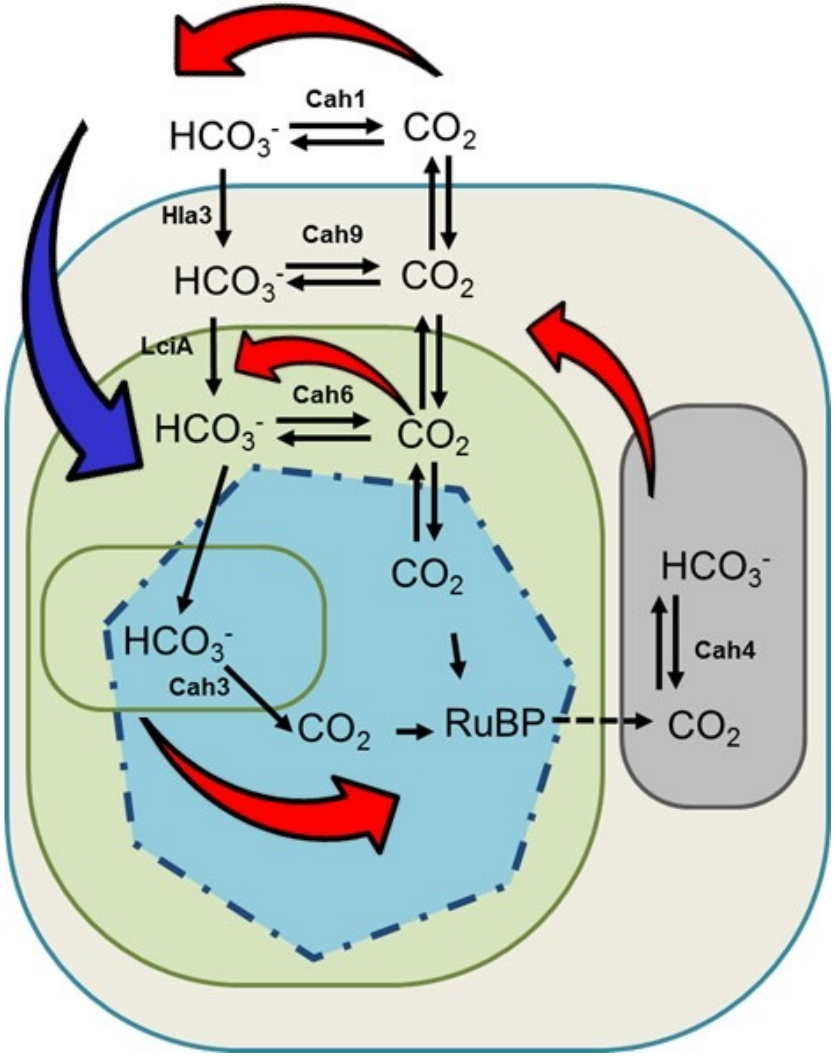
Overexpression mutants in CS:

- ***RG:*** **CAH1, CAH4, HLA3, BCA, LCIA**

- *SN:* SNRK
  Britton T. et al. (In preparation)

- *EG:* PiOxi, P5CS
- *JM:* Guar Gum synthesis
- *CSM:* Increasing Carbon sink
  WSU: 2-Phenylethanol synthesis

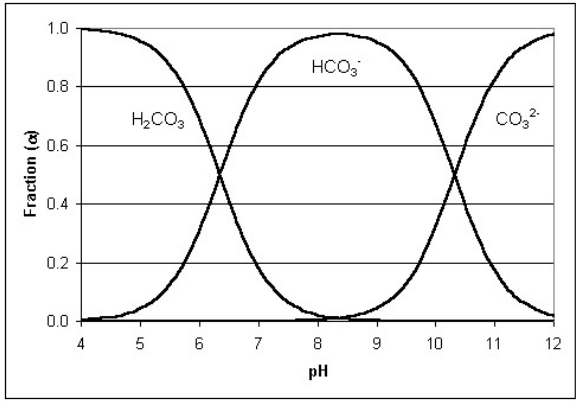
Gene stacking likely required to increase carbon fixation rates



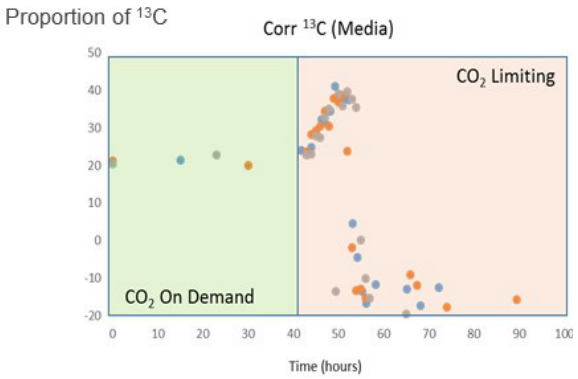
Single overexpression mutants generated in CS:

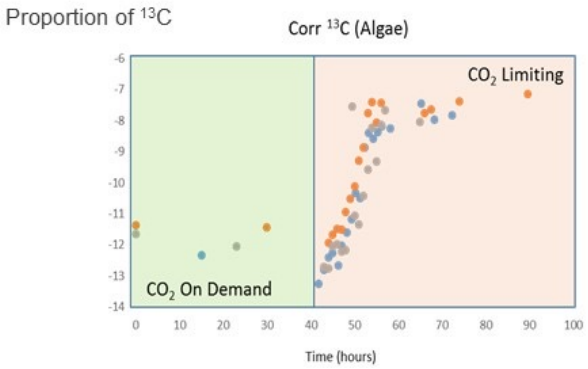
- ***RG:*** **CAH1, CAH4, HLA3, BCA, LCIA**

➢ No positive phenotype observed in single mutants. Likely, multiple gene overexpression required to cross all cellular barriers



Photosynthetic capacity studied by limiting carbon availability

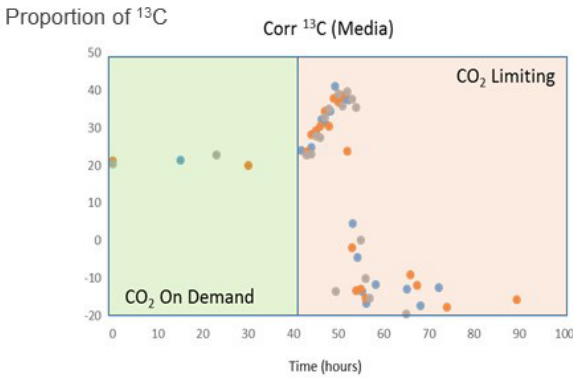


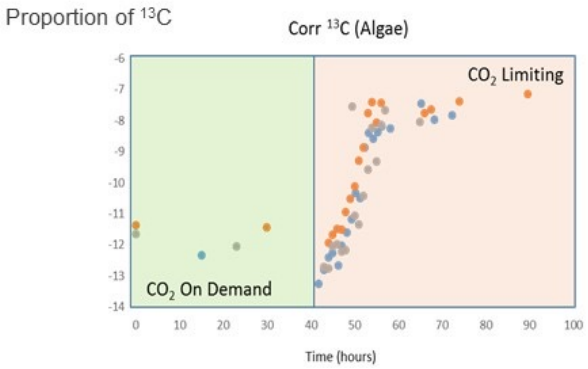


- ^{13}C discrimination tool developed in *N. salina* was implemented for measuring carbon use efficiency in *Chlorella sorokiniana*

Zidenga et al. *Algal Research* (2018)

Photosynthetic capacity studied by limiting carbon availability





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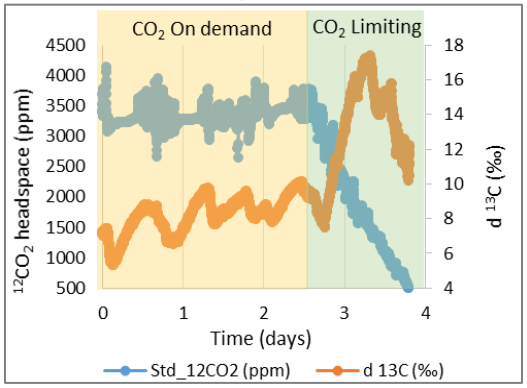
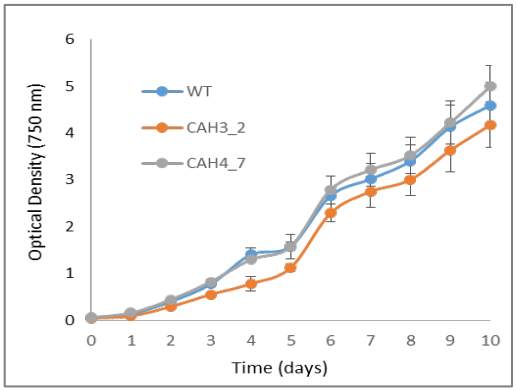
Zidenga et al. *Algal Research* (2018)

… which we are using to evaluate Carbonic anhydrase (CA) mutants in *Nannochloropsis gaditana* (Matt Posewitz, CSM)

Headspace analysis of N. gaditana



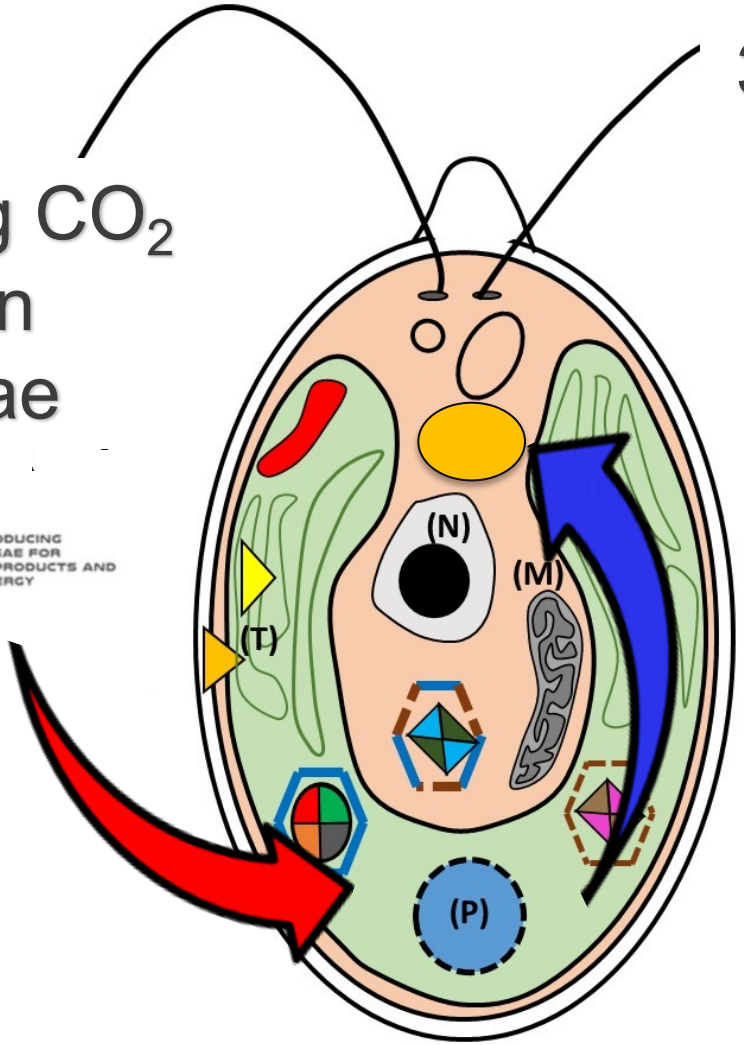
Growth comparison of CA mutants vs WT



Presentation Outline

1) Increasing CO_2 fixation in microalgae

PACE PRODUCING ALGAE FOR COPRODUCTS AND ENERGY



3) Customized PHA production in cyanobacteria

LDRD

Los Alamos NATIONAL LABORATORY EST. 1943

2) Increasing carbon storage compounds in microalgae

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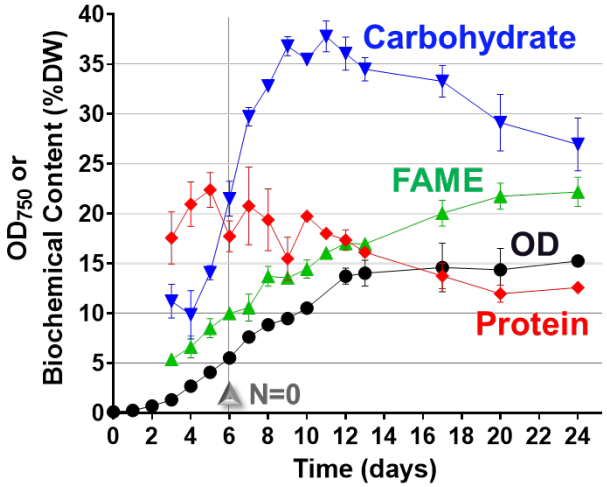
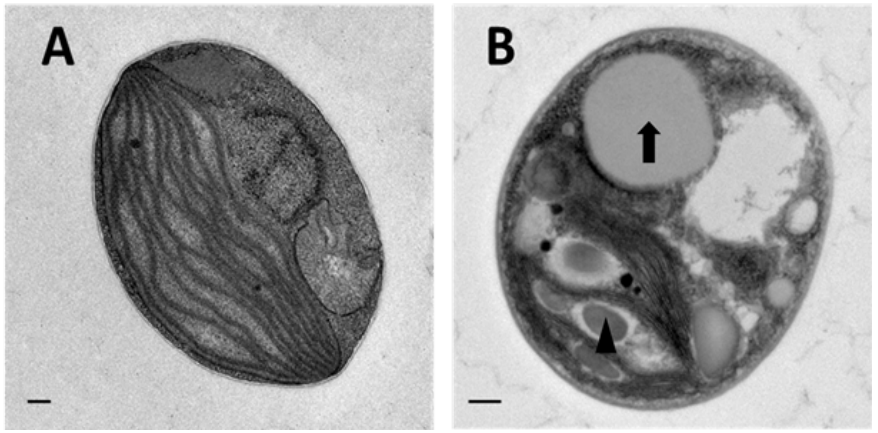
Multiscale Characterization: Increasing carbon storage molecules in algal biomass

- Algae accumulate carbon storage compounds (lipids, carbohydrates) after Nitrogen depletion



P. soloecismus



Gonzalez-Esquer CR et al. *Algal Research*

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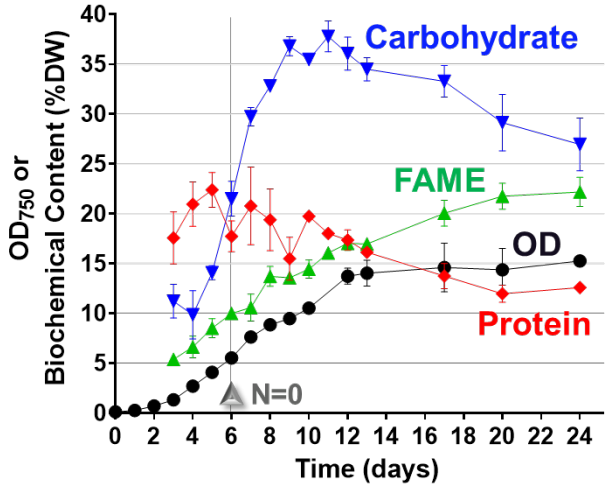

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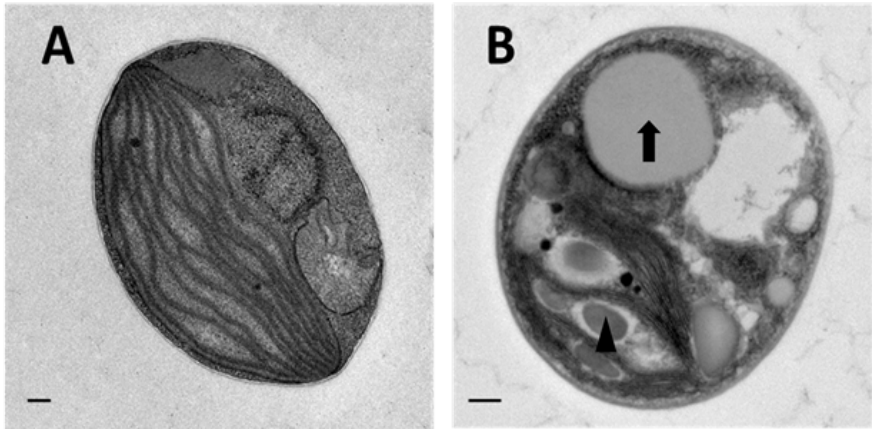
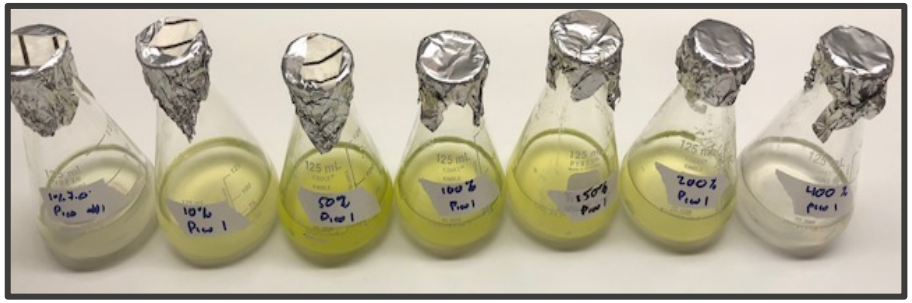
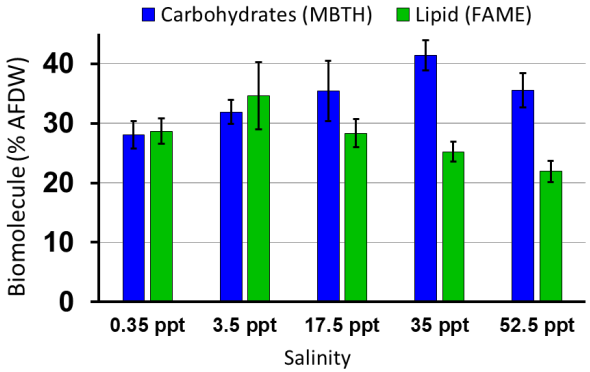


- Proportion of lipids vs carbohydrates may vary under culture conditions

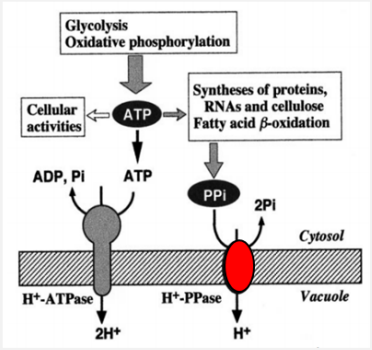


Gonzalez-Esquer CR et al. *Algal Research*



Increasing Carbon Storage in Transgenic Algae

Overexpression of a H^+-pumping pyrophosphatase (AVP1)

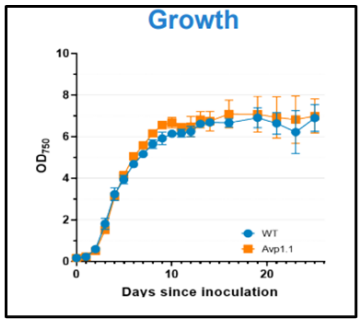


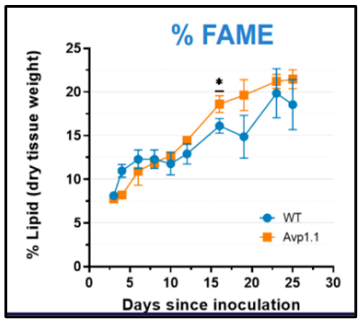
Maeshima, M. (2000).

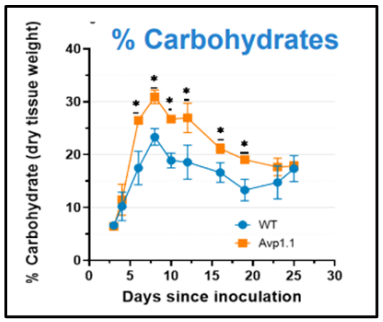
- **Avp1 in plants:**
  - PP_i homeostasis (metabolic regulation)
  - Creates H^+ gradient for ATP synthesis
  - Phloem loading/sucrose transport
- **Avp1 overexpression:**
  - Increases biomass, salt tolerance, and drought tolerance in crops like cotton, tomato, rice, and wheat.

➢ ***Avp1 overexpression effect unknown in algae***

- *AVP1 overexpression increases carbohydrates under N deplete conditions*

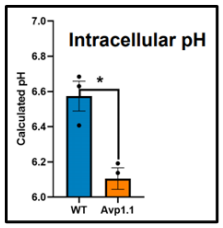


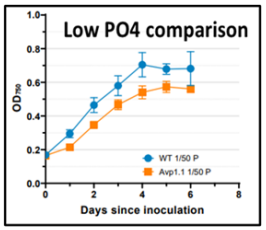




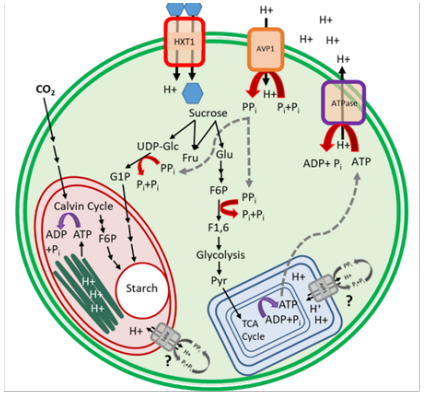
K Wright, R. Gonzalez, T. Dale

- *AVP1 overexpression decreases intracellular pH and diminishes growth on low PO_4 conditions*





- *AVP1 overexpression improves biochemical composition (increased C storage) of algal biomass, likely due to PP_i synthesis (provoking an intracellular P-limiting condition) .*



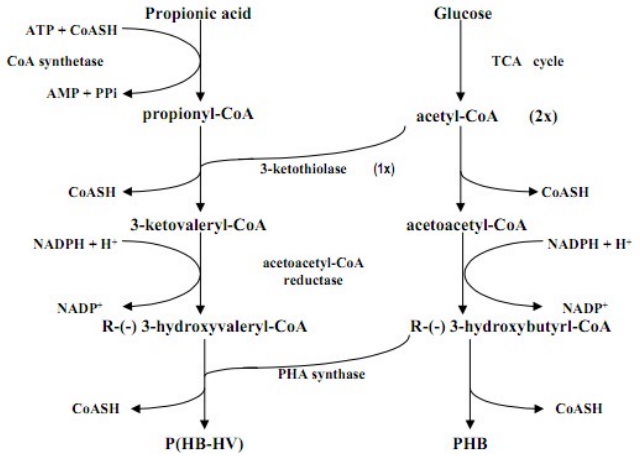
1

BioManiac: Can we leverage biodiversity to match/increase performance of chemically developed materials?

We use **Polyhydroxyalkanoates** (PHA) as case study. PHAs are carbon storage molecules with can be used as bioplastic precursors.

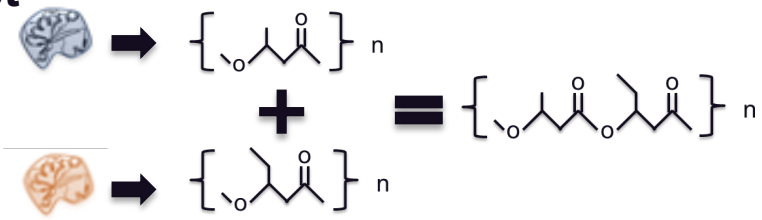
PHA cyanobacteria ≠ PHA heterotrophic bacteria

Metabolism

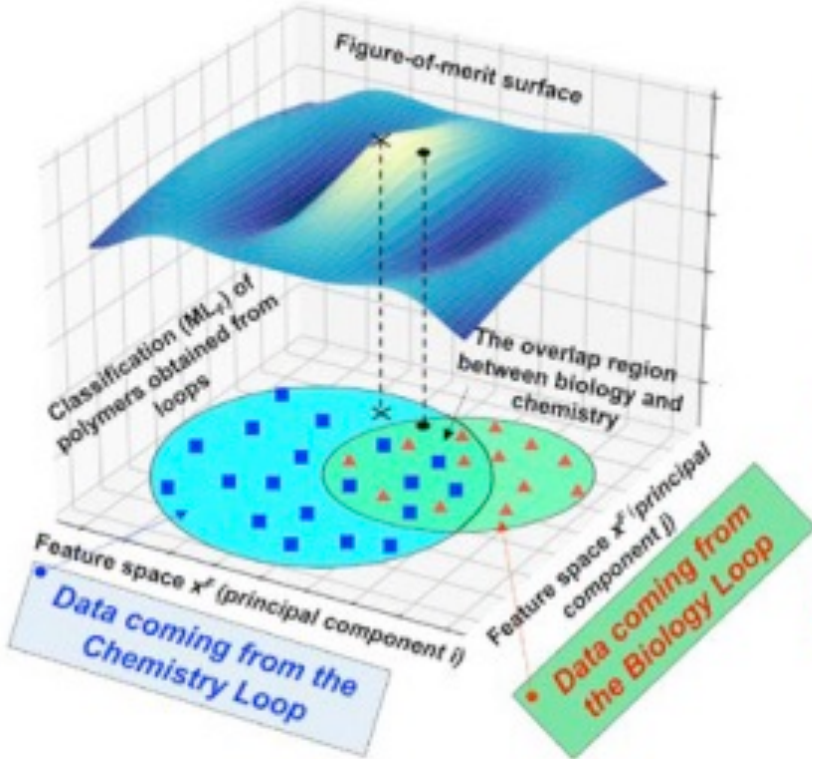


(Ojumu & Solomon, 2004)

Biocatalyst

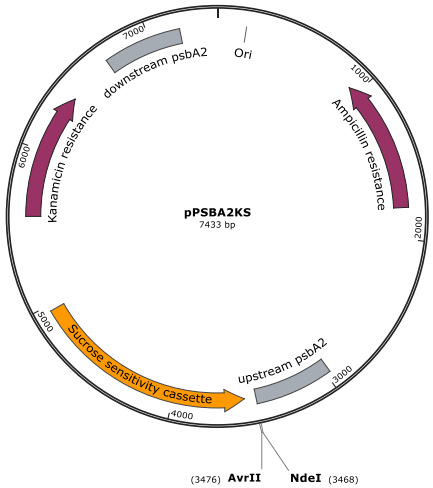


Machine Learning

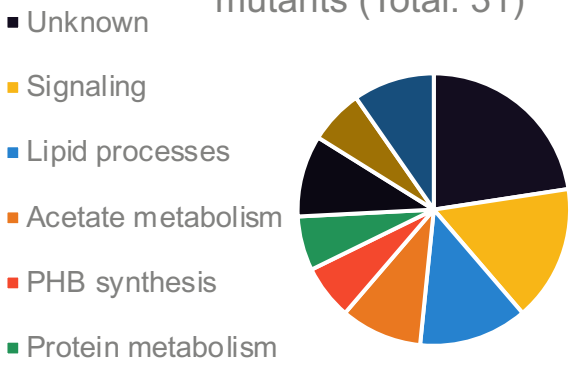


Assessment of ML-Predicted Genes by Overexpression

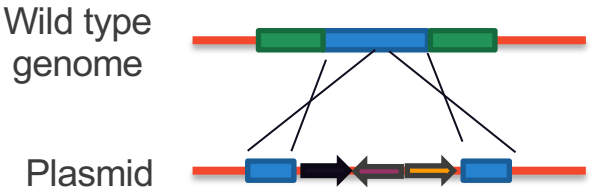
- Gene targets were synthesized into Psba2KS vector and transformed into PCC 6803



Synechocystis sp. PCC 6803 mutants (Total: 31)



- PCR screening of genomic integration



Testing mutant genome

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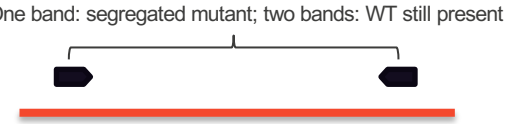
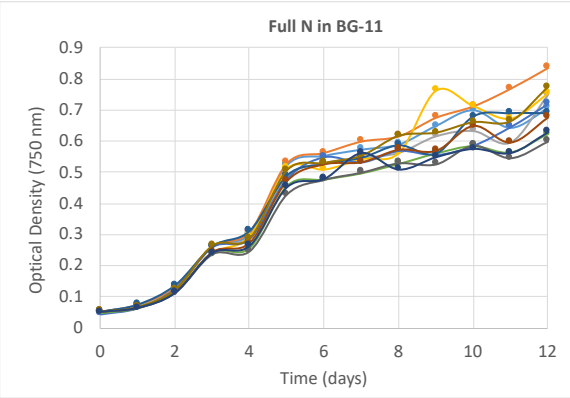
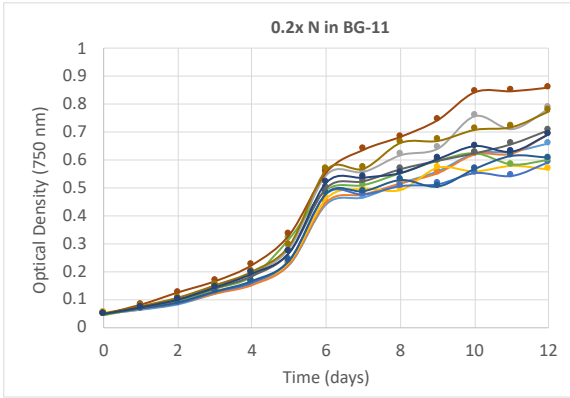


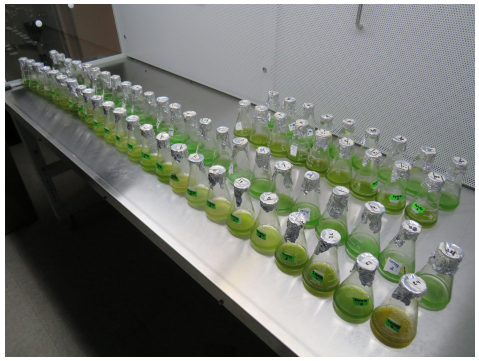
Validation (CyanoGEBA-ML in PCC 6803)

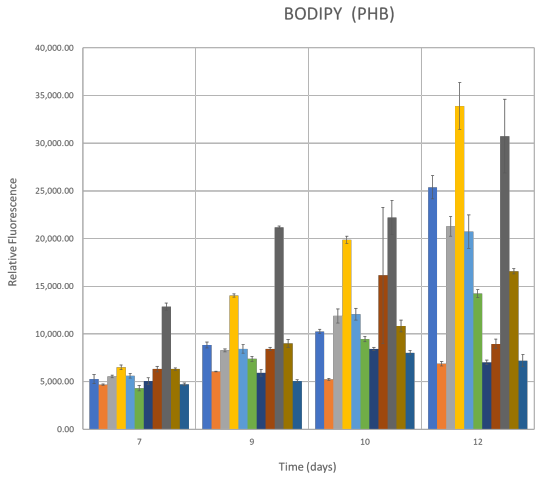
- Growth phenotypes in two different culture conditions





- PHB accumulation via BODIPY/Flow cytometry





Concluding remarks

- LANL is at the forefront of genetic engineering of microalgae
  - We have developed genetic engineering toolboxes for many strains, including *Picochlorum soloecismus, Nannochloropsis salina, Chlorella sorokiniana*
  - We have implemented such toolboxes for generating mutants with favorable phenotypes

- We have the opportunity to integrate metabolic features from different species (cyanobacteria>microalgae>plants) into a synthetic biology discovery and developmental platform
  - We can leverage the cyanobacterial metabolic “simplicity” to engineer complex organisms, i.e. for the production of renewable polymers
  - Unrivaled ‘omics and machine learning scientific collaboration